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Growth and survival of *Puntius conchonius* (Hamilton, 1822) cultured in a Biofloc system.

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ABSTRACT

Rosy barb (*Puntius conchonius*), is an Asia specie that was adapted to culture conditions in our country. Morelos State in México is one of principally national producers of this organism. Biofloc system is a new culture technique that allows development of microbial flocs, formed from incorporation of an external carbon source and that promotes growth of microalgae, protozoans, rotifers and nematodes. To test this technique, eight circular tubs of 200 L were used, in which 30 juvenile organisms of *Puntius conchonius* were placed, with an initial average length of 5.0 ± 0.95 cm and an average weight of 4.2 ± 1.08 g. They were fed with a trout diet (60% of protein) and it was added molasses with rice powder as an external carbon source, guaranteeing a relation C/N 15:1, considering 10% of their body weight and adjusting quantity every 15 days, during 12 weeks. It was observed that with Biofloc treatment, fish reached a weight of 2.45 g, with a length of 5.276 cm and a high of 1.356 cm, higher that fish cultured without Biofloc. Only in width measurement, Biofloc treatment was smaller. ANOVA analysis showed significant differences (P<0.05) between treatments only in length. For all above, use of Biofloc as protein source that are added to diet of *P. conchonius* is important, because it improves growth in size, weight and survival, and provides live food that this fish need to avoid high mortality that is present in controlled cultures.

Key words: *Puntius conchonius*, Biofloc, growth rates, condition factor.

RESUMEN

El barbo rosi (*Puntius conchonius*), especie originaria de Asia, ha logrado adaptarse a las condiciones de cultivo en nuestro país. El Estado de Morelos es uno de los principales productores, a nivel nacional, de este organismo. Una nueva técnica de cultivo es el sistema Biofloc, el cual permite el desarrollo de flóculos microbianos formados a partir de la incorporación de una fuente de carbono externa y que promueve el crecimiento de microalgas, protozoarios, rotíferos y nematodos. Para probar dicha técnica se pusieron ocho tinas circulares de 200 L, en las cuales se pusieron 30 organismos juveniles de *Puntius conchonius*, con una longitud inicial promedio de 5.0 ± 0.95 cm y un peso promedio de 4.2 ± 1.08 g. Se les suministró una dieta para trucha (60% de proteína) y se adicionó melaza+polvillo de arroz como fuente externa de carbono, garantizando una relación C/N 15:1, considerando el 10% de su masa corporal y ajustando la cantidad cada 15 días, durante 12 semanas. Se observó que en el tratamiento con Biofloc, los peces alcanzaron un peso de 2.45 g, una longitud de 5.276 cm y altura de 1.356 cm, mayor que en los peces cultivados sin Biofloc. Solo en la medida de ancho, el tratamiento con Biofloc fue menor. El análisis de varianza señaló diferencias significativas (P<0.05) entre los tratamientos solo con respecto a la variable longitud. Por todo lo anterior, la utilización del Biofloc como fuente de proteínas que se añaden a la dieta de *P. conchonius* es importante, ya que mejora el crecimiento en talla y peso, mejora la supervivencia y provee el alimento vivo que necesitan estos peces para evitar la alta mortalidad que se da en cultivos controlados.

Palabras clave: *Puntius conchonius*, Biofloc, tasas de crecimiento, factor de condición.

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INTRODUCTION

In México, about 43 million ornamental fish are traded, mainly of fresh water (SAGARPA 2012). Morelos State is principally national producer with 20 million organisms annually, approximately in 80 hectares (Diaro Oficial de la Federación 2012), where 62 distinct species are produced, in which rosy barb (*Puntius conchonius*) stands out, a specie native from Asia, which has managed to adapt to culture conditions in our country.

Among advantages this ornamental fish presents (peaceful and active species), their striking aesthetic by what it has become a specie of high demand in ornamental fish market (Mir and Mir 2012). This fish is cultured in intensive and semi-intensive systems and fed with artificial diets. Nevertheless, due to production conditions with high densities and limited water quality, organisms are under constant stress, which causes low growth rates and constant mortality (Akinbowale et al. 2006). Therefore, there is a continuous search for food alternatives that allow to improve their growth and resist environmental and man-handle variations.

A very interesting strategy is application of Biofloc culture systems, which allows development of microbial flocs formed from incorporation of an external carbon source as molasses, rice bran, wheat bran, among others (Avnimelech 2006). Associated to these flocs, it is promoted growth of microalgae, protozoans, rotifers, and nematode, which serve as *in situ* natural food and can be used by cultured species (De Schryver et al. 2008; Ekasari et al. 2010). For all above, aim of this work is to evaluate survival and growth rate of *P. conchonius* cultured in a Biofloc system.

MATERIAL AND METHODS

Experimental design and culture conditions

For this study, eight glass fiber tubs of 200 L were used. Four of them were used for Biofloc treatments and other four for control test. In each tube, 30 juvenile organisms of *P. conchonius* were placed, with an average initial length of 5.0 ± 0.95 cm and an average weight of 4.2 ± 1.08 g (Fig. 1). The organisms were fed with commercial food for trout (60% of protein) considering 10% of their body weight and adjusting quantity every 15 days. In Biofloc treatments it was added molasses and rice powder as an external carbon source to promote development of flocs considering calculation requirement as recommended by Emerenciano (2011) and guarantee a relation C/N 15:1. Water was not changed in these tubs and it was maintained constant aeration to avoid sedimentation of flocs. Experimentation test period was 12 weeks.

Survival and growth parameters estimation

To evaluate growth rate of fishes, every 15 days, 10 organisms were weighted in a digital balance

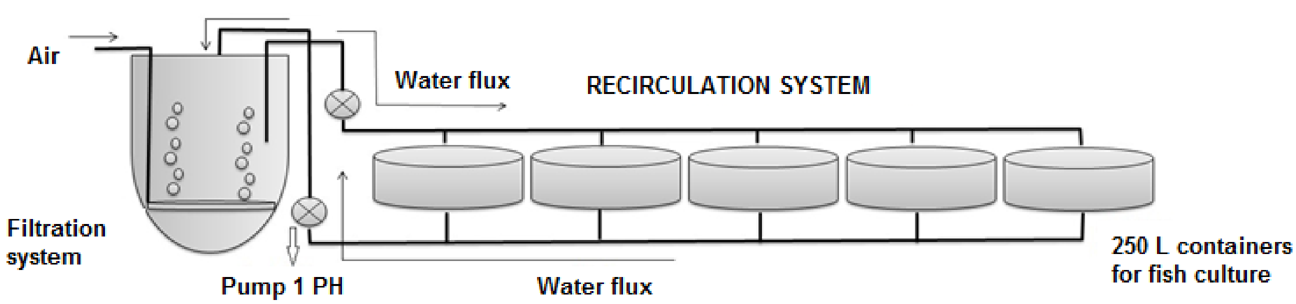

Fig. 1. Experimental system with Biofloc for *P. conchonius*.

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OHAUS (model Scout Pro 400 g with precision of 0.001 g). Total length, high, and width measurements were taken with help of a digital Vernier (0-150 mm with precision of ± 0.5 mm). Fish were captured with nets and anesthetized using a solution of clove oil (5 mL L^{-1}). Daily it was registered mortality in each experimental unit. Survival and growth values were introduced to a data base in Excel 2010. With growth values were made tendency curves.

Processing data

In Excel 2010, it was determined descriptive analysis of biometric values. To know well-being state of organisms in culture test, it was determined condition factor (K) according to mathematical expression (Martínez-Millán 1987):

$$K = \frac{W}{L^3} \times 100$$

Where:
W: Body weight of fish expressed in grams
L: Length expressed in centimeters
3: Isometric growth constant

The increase and gain of weight as well as biomass of *P. conchonius* was calculated through next formulas respectively (Moreno et al. 2000):

$$Wr = K \times Lo$$

Where:
Wr: Weight increase expressed in grams
K: Condition factor
Lo: Initial length expressed in centimeters

$$GP = W_2 - W_1$$

Where:
GP: Weight gain in grams
W_2: Weight in grams at end of period
W_1: Weight in grams at beginning of period

Final Biomass= Average weight x N° of organisms

The absolute growth rate (AGR) was obtained using the next formula (Soriano and Hernández 2002):

$$AGR = \frac{VBf - Vbi}{Tf - Ti}$$

Where:
AGR: Absolute Growth Rate
Vbf = Final biometric variable (length, high, width or weight)
Vbi= Initial biometric variable (length, high, width or weight)
Tf= Final time (days)
Ti= Initial time (days)

The instantaneous growth rate (IGR) was obtained with next formula (Soriano and Hernández 2002):

$$IGR = \frac{LN\ Vbf - LNVbi}{Tf - Ti} \times 100$$

Where:
LN= natural logarithm
Vbf= Final biometric variable
Vbi= Initial biometric variable
Tf= Final time (days)
Ti= Initial time (days)

RESULTS

Mean values and tendency curves

Mean values (±S.D.) of biometric variables considered in this experiment with *P. conchonius* are shown in Table 1 (treatment with Biofloc) and Table 2 (treatment without Biofloc) during 60 days of culture (four samplings every 15 days). Growth tendency curves are shown in Fig. 2 and 3, in which it is observed a second-degree polynomial curve in most of variables. Only variable that presented a third-degree polynomial curve was length with Biofloc treatment.

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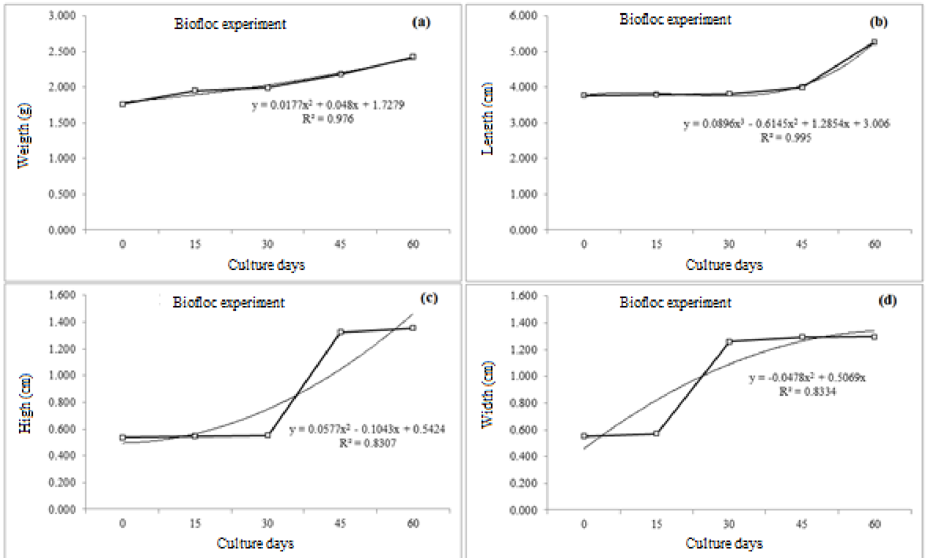

Fig. 2 Growth tendency curves of weight (a), length (b), high (c) and width (d) of *P. conchonius* organisms with Biofloc treatment.

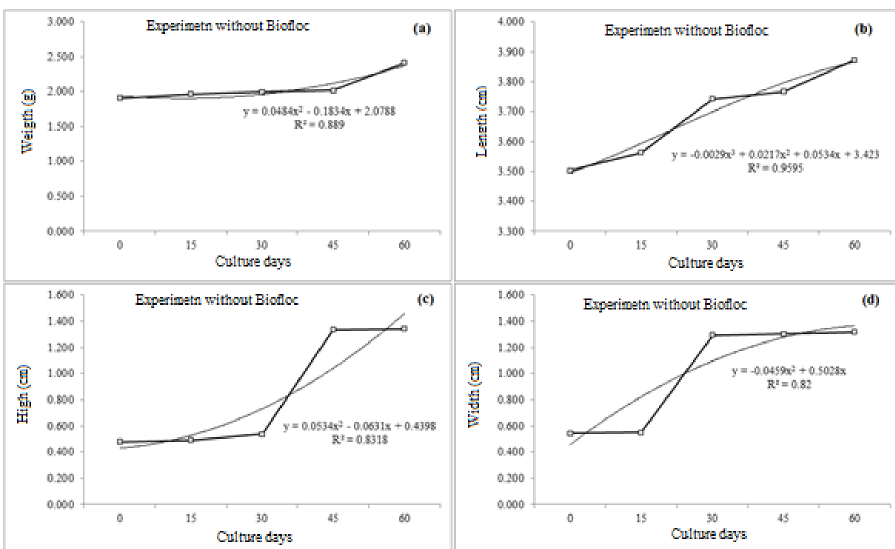

Fig.3. Growth tendency curves of weight (a), length (b), high (c) and width (d) of *P. conchonius* organisms without Biofloc treatment.

Final Biometry

Table 3 shows final biometric variable mean values obtained by each treatment (Biofloc and without Biofloc). Higher values of weight, length and high were observed in organisms of Biofloc system, but width variable was higher in without Biofloc treatment. ANOVA test showed significant

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Table 1. Mean values (±S.D.) of biometric variables of *P. conchonius* in a culture of 60 days with Biofloc.

Culture days	Weight (g)	Length (cm)	High (cm)	Width (cm)
0	1.769	3.777	0.537	0.554
	±0.343	±0.199	±0.075	±0.061
15	1.956	3.792	0.547	0.570
	±0.214	±0.324	±0.059	±0.085
30	1.998	3.815	0.554	1.259
	±0.289	±0.285	±0.056	±0.215
45	2.187	4.004	1.326	1.295
	±0.412	±0.541	±0.122	±0.095
60	2.425	5.276	1.356	1.296
	±0.191	±0.435	±0.104	±0.104

Table 2. Mean values (±S.D.) of biometric variables of *P. conchonius* in a culture of 60 days with Biofloc.

Days of culture	Weight (g)	Length (cm)	High (cm)	Width (cm)
0	1.909	3.502	0.480	0.544
	±0.329	±0.592	±0.099	±0.062
15	1.964	3.564	0.492	0.548
	±0.417	±0.282	±0.056	±0.119
30	1.996	3.743	0.536	1.293
	±0.295	±0.408	±0.040	±0.124
45	2.016	3.767	1.336	1.304
	±0.316	±0.216	±0.085	±0.074
60	2.416	3.873	1.344	1,319
	±0.568	±0.386	±0.074	±0.125

Table 3. Mean values of final biometry (60 days of culture) of organisms in both treatments (Biofloc and without Biofloc).

Treatment	Weight (g)	Length (cm)	High (cm)	Width (cm)
With Biofloc	2.425	5.276	1.356	1.296
	±0.191	±0.435	±0.104	±0.104
Without Biofloc	2.416	3.873	1.344	1.319
	±0.568	±0.386	±0.074	±0.125

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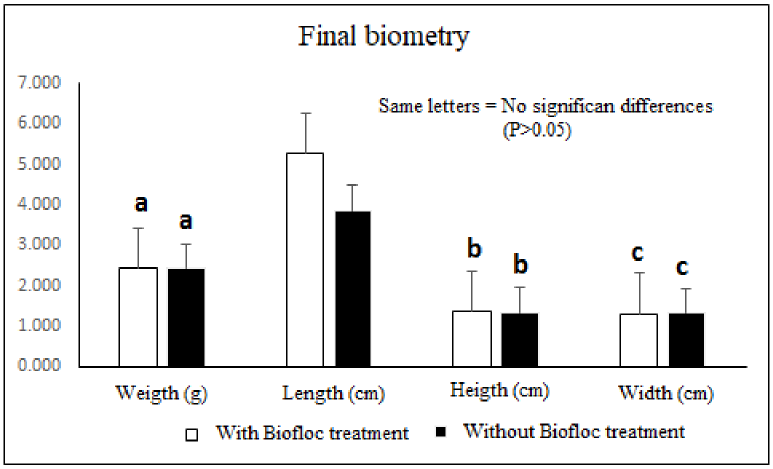

Fig. 4. Variance analysis of final biometry of considered biometric variables in both treatments.

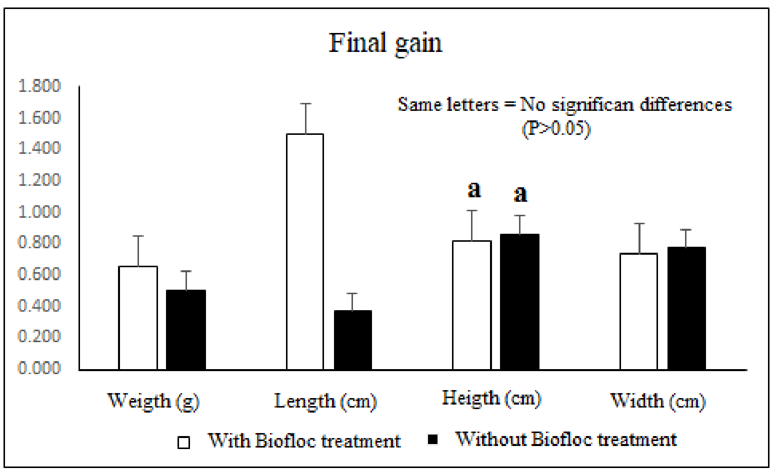

Fig 5. Variance analysis of the gains in biometric variables considered in both treatments

differences (P<0.05) between treatments only in length variable (Fig.4).

Gains of biometric variables

Table 4 shows mean values (±S.D.) of final gain values obtained in both treatments, as well as its relation in percentage. It can be observed a higher gain of weight and length in Biofloc treatment and higher gain in high and width in treatment without Biofloc. In Fig. 5 it is presented ANOVA test of final gain between treatments. It was observed that variables present significant differences between treatments (P<0.001), except in high values.

Absolut growth rate (AGR)

Mean values (±S.D.) of increase in considered biometric values are presented in Table 5. It was obtained higher values of daily increase in weight and length in treatment with Biofloc, and high

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Table 4. Mean values (±S.D.) of final gain obtained by organisms in both treatments. Percent values are added.

Treatment	Weight (g)	Length (cm)	High (cm)	Width (cm)
With Biofloc	0.656	1.499	0.819	0.742
	±0.065	±0.190	±0.091	±0.024
Without Biofloc	0.507	0.371	0.863	0.775
	±0.070	±0.050	±0.060	±0.060
	Weight (%)	Length (%)	High (%)	Width (%)
With Biofloc	37.089	39.683	152.485	133.973
	±9.700	±8.690	±24.920	±31.790
Without Biofloc	26.579	10.589	179.834	142.548
	±5.860	±1.590	±13.870	±25.100

Table 5. Mean values (±S.D.) of AGR of considerate variables between experimental treatments.

Treatment	Weight (g)	Length (cm)	High (cm)	Width (cm)
With Biofloc	0.0109	0.0250	0.0137	0.0124
	±0.0010	±0.0020	±0.0010	±0.0010
Without Biofloc	0.0085	0.0062	0.0144	0.0129
	±0.0005	±0.0002	±0.0040	±0.0030

and width in treatment without Biofloc, even though, ANOVA test does not show significant differences with these two last variables between treatments (Fig. 6).

Instantaneous growth rate (IGR)

Table 6 shows increase mean values (±S.D.) in considerate biometric variables. Higher values of weight and length gain were obtained in treatment with Biofloc; but variables high and width were higher in treatment without Biofloc. Even though, ANOVA analysis points out significant differences between all considerate biometric variables (Fig. 7).

DISCUSSION

Biofloc can substitute commercial food used in culture of fish and it has been observed growth and wealth being of several species like *Oreochromis mossambicus* (Avnimelech 2007); also of *Macrobrachium rosenbergii* (Asaduzzamann et al. 2008) and *Litopenaeus vannamei* (Buford et al. 2004 and Xu et al. 2012 a,b). Wang et al. (2015) found, with other ornamental fish *Carassius auratus*, that with high values of C/N relation (20:1 and 25:1) in a Biofloc system, gain values in weight (110.17±4.99) specific growth (1.33±0.04), were better comparing to control treatment (82.52+-3.33, 1.07±0.033

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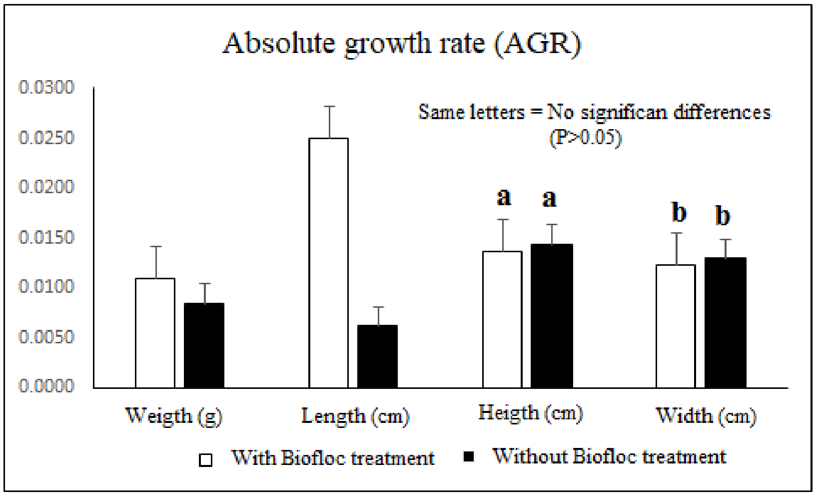

Fig. 6. Variance analysis of AGR of considerate biometric variables between both experimental treatments.

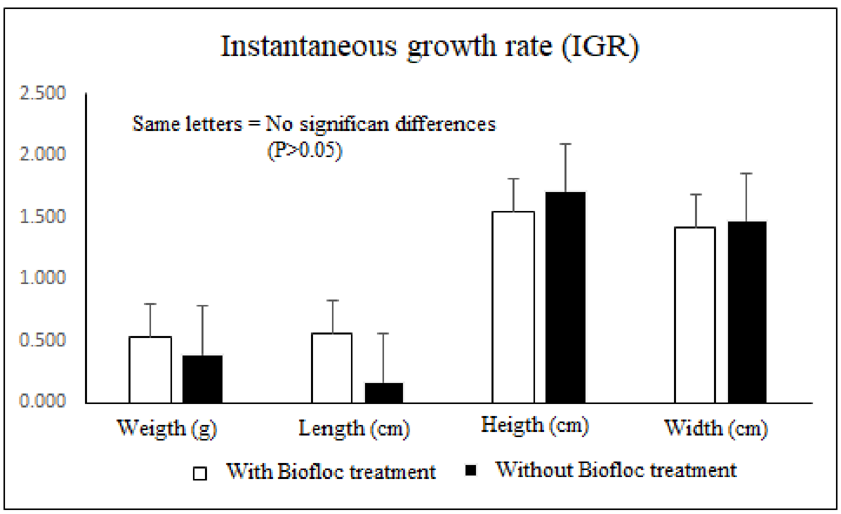

Fig. 7. IGR variance analysis of considerate biometric variables between experimental treatments.

respectively). In this study, *P. conchonius* cultured with Biofloc, there were significant differences between gain of weight (37.89%) and length (39.68%) with respect to control treatment. Crab et al. (2009) mentions a higher gain in mean weight (0.29±0.03 g) in fish cultured in water tank with Biofloc than control. Kuhn et al. (2009) reports that diet with flocs of Biofloc can increase growth of fish in a 65.1% above average growth in systems with control diets. Azim and Little (2008) register 45%

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Table 6. IGR mean values (±S.D.) of considerate variables between both experimental treatments.

Treatment	Weight (%)	Length (%)	High (%)	Width (%)
With Biofloc	0.526	0.557	1.544	1.417
	±0.020	±0.070	±0.040	±0.070
Without Biofloc	0.393	0.168	1.715	1.477
	±0.039	±0.016	±0.071	±0.014

more fish production in water tanks with Biofloc system than control tanks. Which was proved in this experiment, because fish with Biofloc treatment increased its weight 37.089% and 39.683% in total length from the beginning of experiment and were higher values in a 28.33% regarding to treatment without Biofloc (26.579% and 10.589% respectively).

The relation carbon:nitrogen (15:1) used in this experiment, allowed to obtain necessary heterotrophic bacteria to maintain controlled nitrites, nitrates and ammonium levels, because water change was minimum throughout experiment and fish were in good health. Previous studies with fish farmed with Biofloc system like Hargreaves (2006), Asaduzzaman et al. (2008), Ballester et al. (2010), and Wang et al. (2015), mentions that maintaining high levels of C:N (10 to 20) promotes proliferation of Biofloc in ponds, and it also promotes growth of heterotrophic microorganisms that consume nitrogenous compounds, ammonium, nitrates and organic matter that allows increase of Biofloc quantity in system (Xia et al. 2012; Wang et al. 2015). Manipulation of C/N relation in system by adding carbohydrates, significantly reduces inorganic nitrogen concentration in water column and total nitrogen kin sediments (Azim and Little 2008), allowing to have a good water quality in cultures and therefore well-being in cultured fish. In high values of C/N relation, heterotrophic microorganisms dominate over autotrophic microorganisms and assimilate total of ammoniacal nitrogen, nitrites and nitrates that produce cellular protein that can be use as external source of food supplement for fish (Buford 2003; Buford and Lorenzen 2004; Souza et al. 2014).

There are several studies bout *P. conchonius* and its relation weight-size and factors condition carried out in natural environment in rivers of India (Mir and Mir 2012), also studies about type of food, feeding habits and reproductive biology (Gupta 2015), as well as incorporation of rose petals flour in diet to increase coloration in this fish (Pailan et al. 2012). In Mexico, there is Dominguez and Martínez (2016) study, which evaluated growth of *P. conchonius* under three treatments, one commercial and two experimental of low cost and processing: earthworm (*Eisenia foetida*) and mealworm (*Tenebrio molitor*). They mention that with *E. foetida* it was obtained higher values of weight (0.98±0.07 g) and length of 3.815±0.285, while when cultured with Biofloc, was obtained a higher weigh (51% more) and length (21% more) than ones fed only with earthworm, mealworm, and commercial food.

For all above, utilization of Biofloc as a protein source that add to *P. conchonius* diet is important, because it improves growth on size and weight, improves survival and provides live food that this fish needs to avoid high mortality that occur in controlled cultures. The Biofloc system for *P. conchonius* production can be used to help producers of ornamental fish and therefore increase fish production for aquarium-keeper and without

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dependence on external live foods that are not always available and which increase food costs.

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